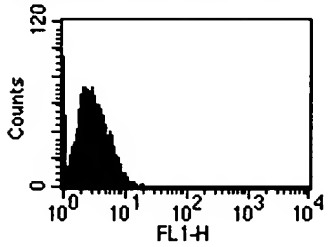
FIGURE 1

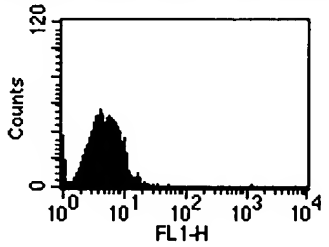
Y1251F IGF-I Receptor Cells

Insulin Receptor Cells

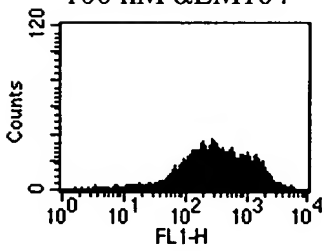
Goatαmouse-IgG-FITC only



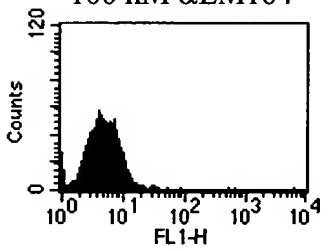
Goatαmouse-IgG-FITC only



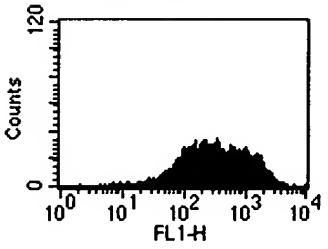
100 nM αEM164



100 nM αEM164



100 nM α1H7



100 nM αIR

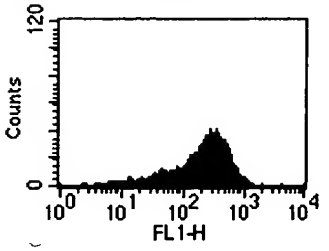


FIGURE 2

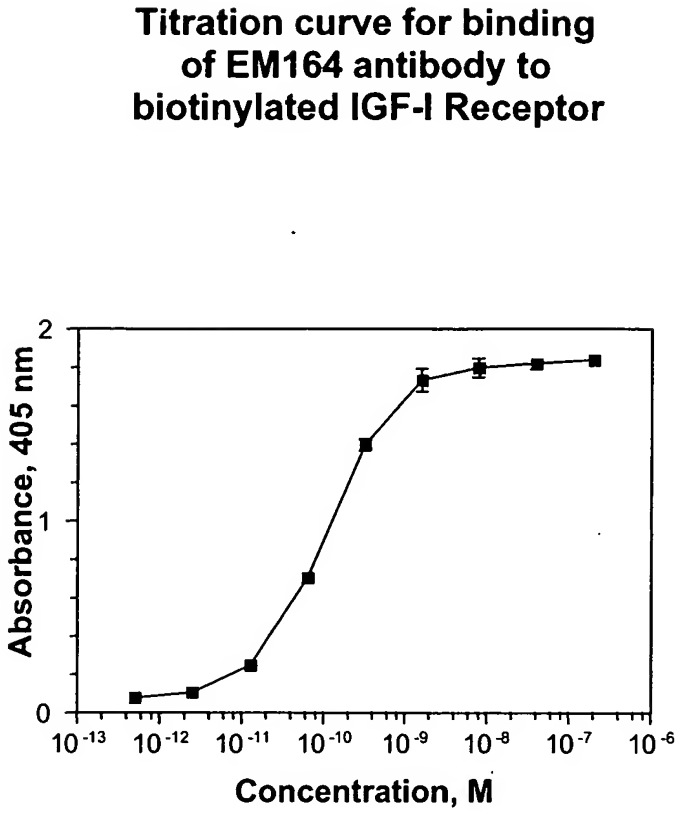

Titration curve for binding of EM164 antibody to biotinylated IGF-I Receptor
Absorbance, 405 nm
2
1
0
10-13
10-12
10-11
10-10
10-9
10-8
10-7
10-6
Concentration, M

FIGURE 3

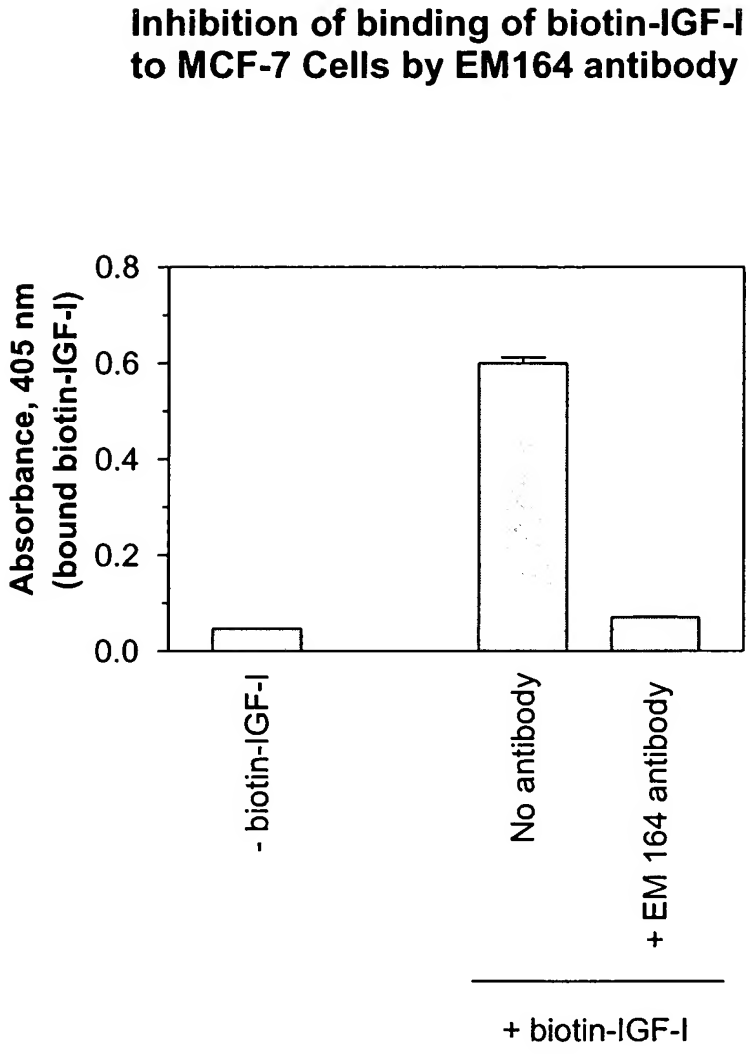

Inhibition of binding of biotin-IGF-I to MCF-7 Cells by EM164 antibody
Absorbance, 405 nm (bound biotin-IGF-I)
0.8
0.6
0.4
0.2
0.0
- biotin-IGF-I
No antibody
+ EM 164 antibody
+ biotin-IGF-I

FIGURE 4

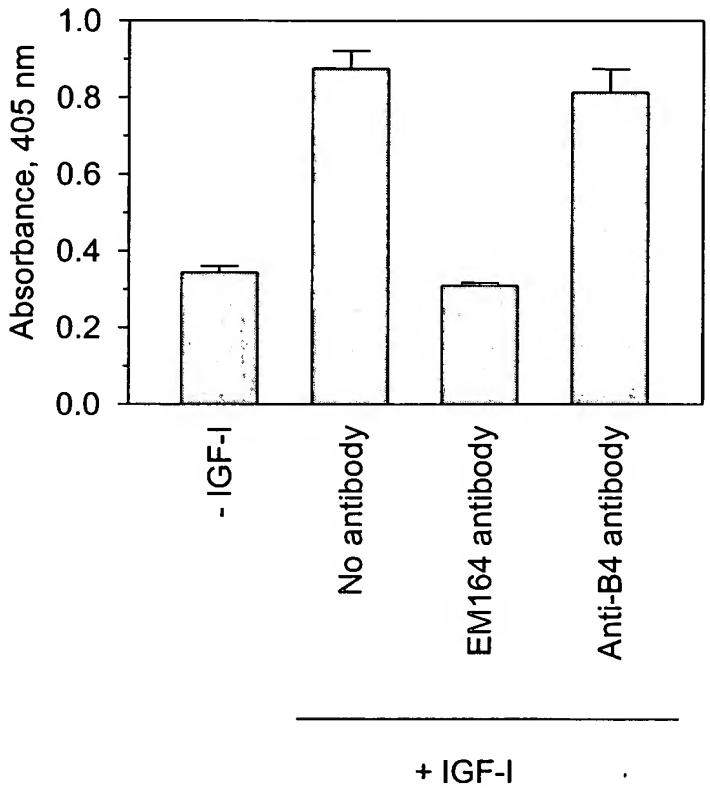

Inhibition of IGF-I-Stimulated Autophosphorylation of IGF-I Receptor in MCF-7 Cells by EM164 Antibody
Absorbance, 405 nm
1.0
0.8
0.6
0.4
0.2
0.0
- IGF-I
No antibody
EM164 antibody
Anti-B4 antibody
+ IGF-I

FIGURE 5

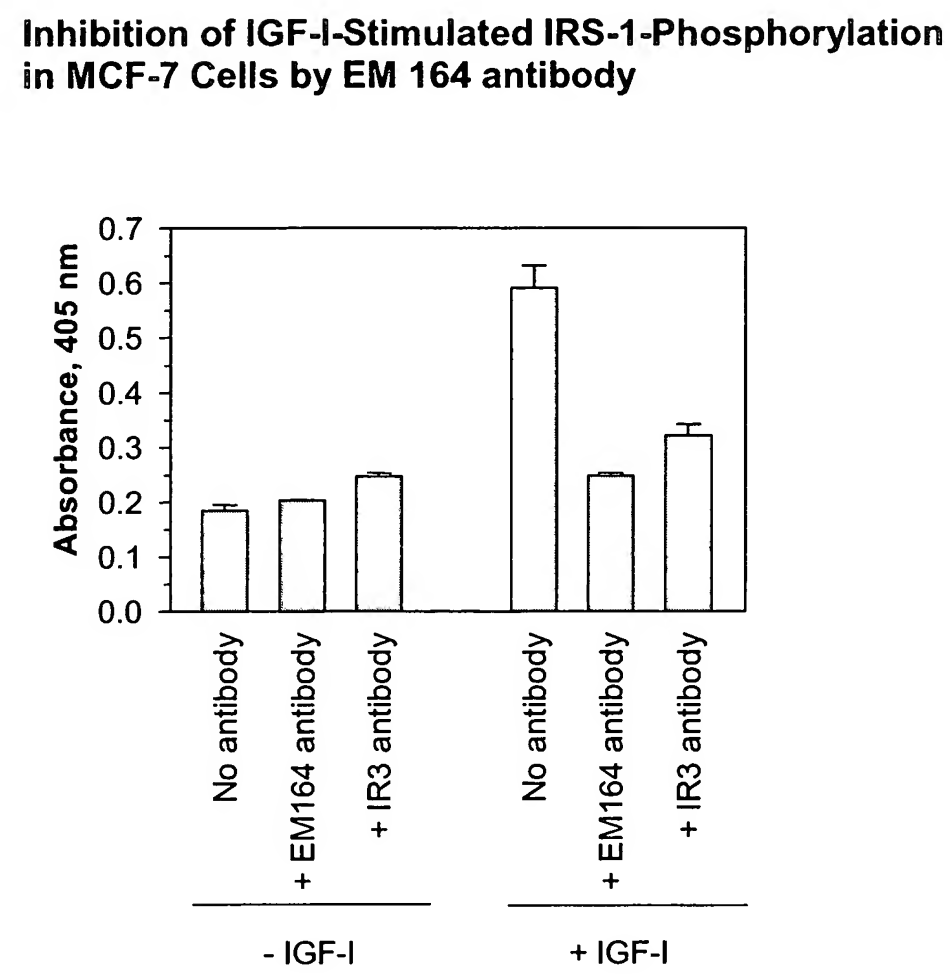

Inhibition of IGF-I-Stimulated IRS-1-Phosphorylation in MCF-7 Cells by EM 164 antibody
Absorbance, 405 nm
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
No antibody
+ EM164 antibody
+ IR3 antibody
No antibody
+ EM164 antibody
+ IR3 antibody
- IGF-I
+ IGF-I

FIGURE 6

Inhibition of IGF-I-Stimulated Signal Transduction in SaOS-2 Cells by EM164 Antibody

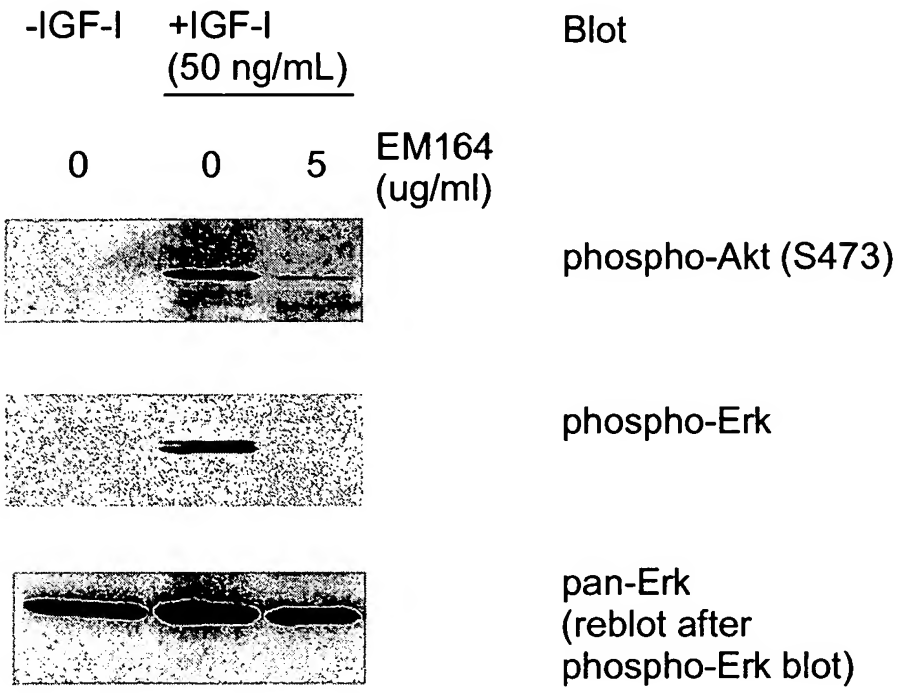


FIGURE 7

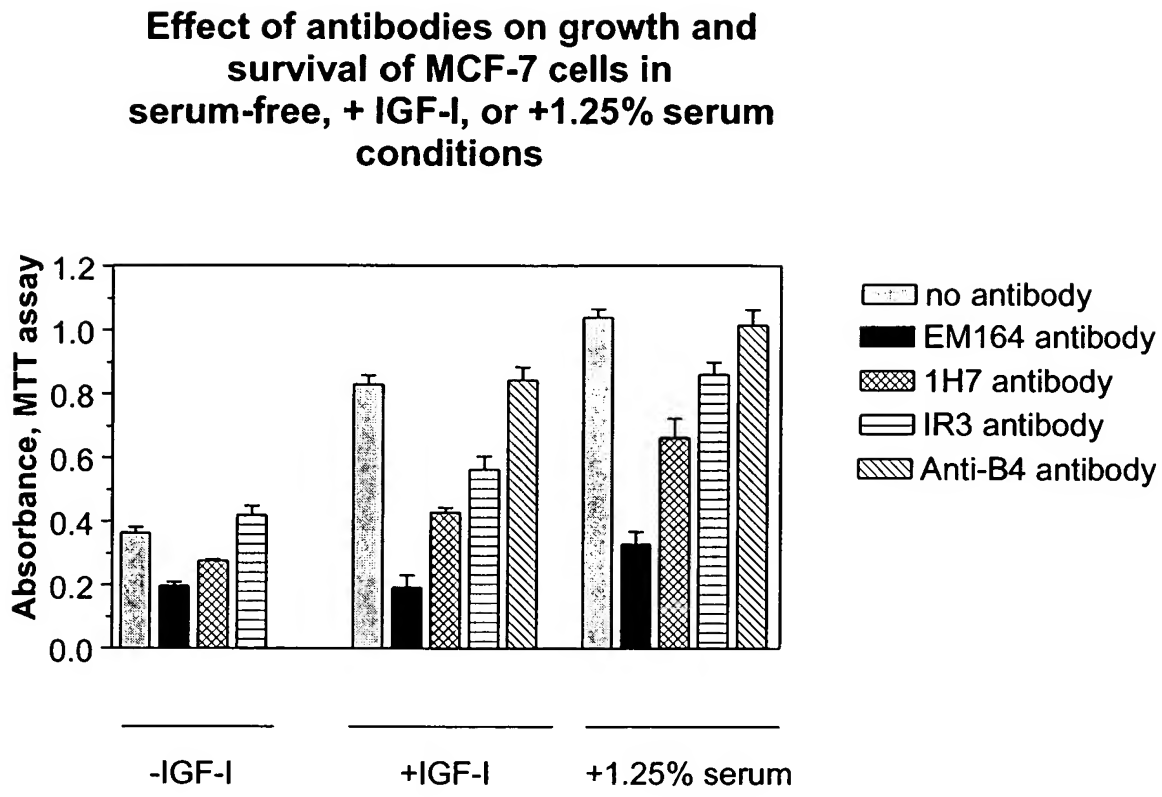

Effect of antibodies on growth and survival of MCF-7 cells in serum-free, + IGF-I, or +1.25% serum conditions
Absorbance, MTT assay
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-IGF-I
+IGF-I
+1.25% serum
no antibody
EM164 antibody
1H7 antibody
IR3 antibody
Anti-B4 antibody

FIGURE 8

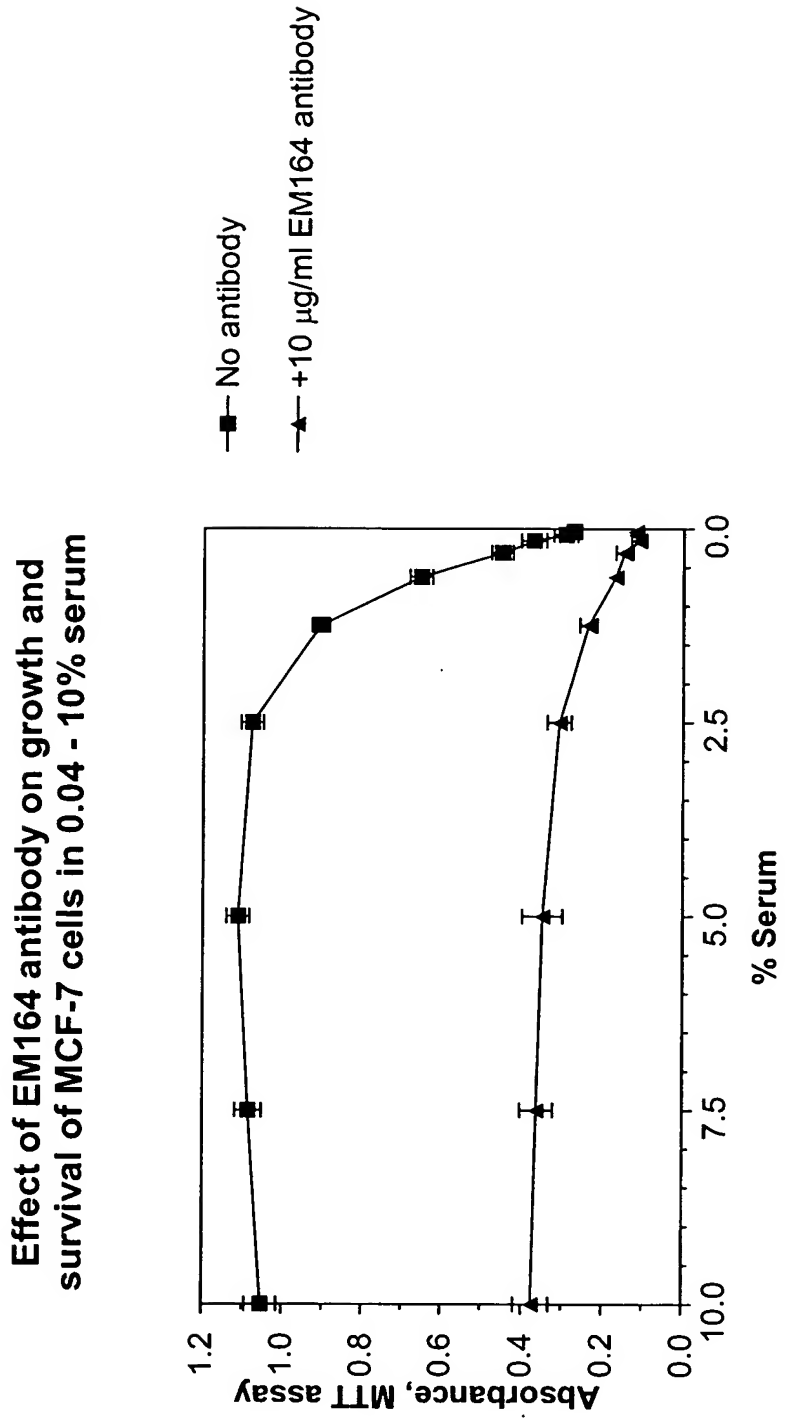

Effect of EM164 antibody on growth and survival of MCF-7 cells in 0.04 - 10% serum
No antibody
+10 µg/ml EM164 antibody
Absorbance, MTT assay
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10.0
7.5
5.0
2.5
0.0
% Serum

FIGURE 9

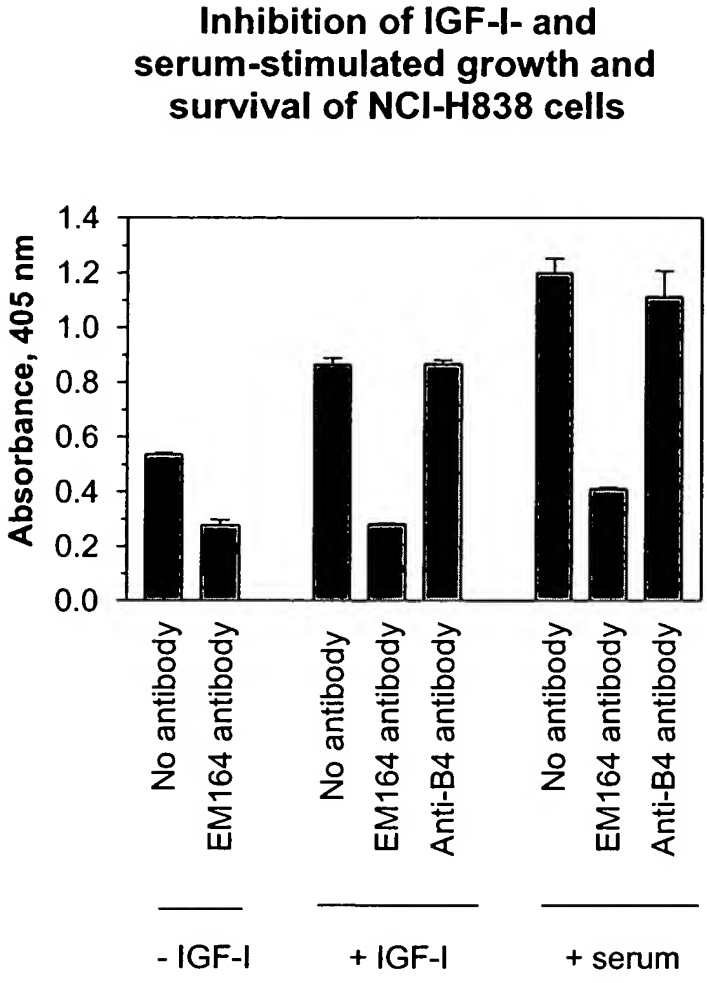
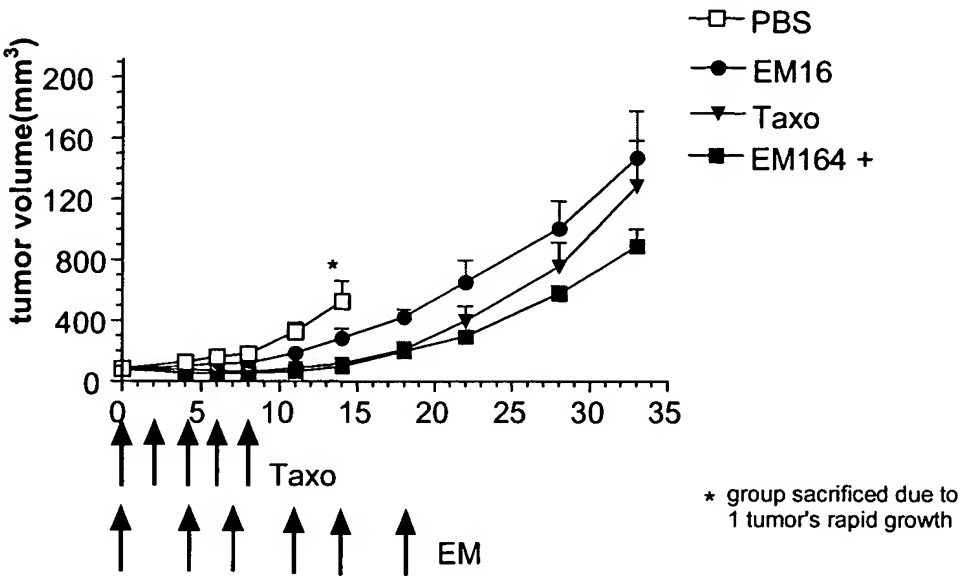

Inhibition of IGF-I- and serum-stimulated growth and survival of NCI-H838 cells
Absorbance, 405 nm
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
No antibody
EM164 antibody
No antibody
EM164 antibody
Anti-B4 antibody
No antibody
EM164 antibody
Anti-B4 antibody
- IGF-I
+ IGF-I
+ serum

FIGURE 10

Effect of treatment with EM164 antibody, or taxol, or a combination of EM164 antibody and taxol, on growth of Calu-6 lung cancer xenograft in mice



days

FIGURE 11

Competition of binding of humanized EM164 antibody (version 1.0) to immobilized biotinylated IGF-I receptor by murine EM164 antibody (1.06 to 10.6-fold molar concentration range)

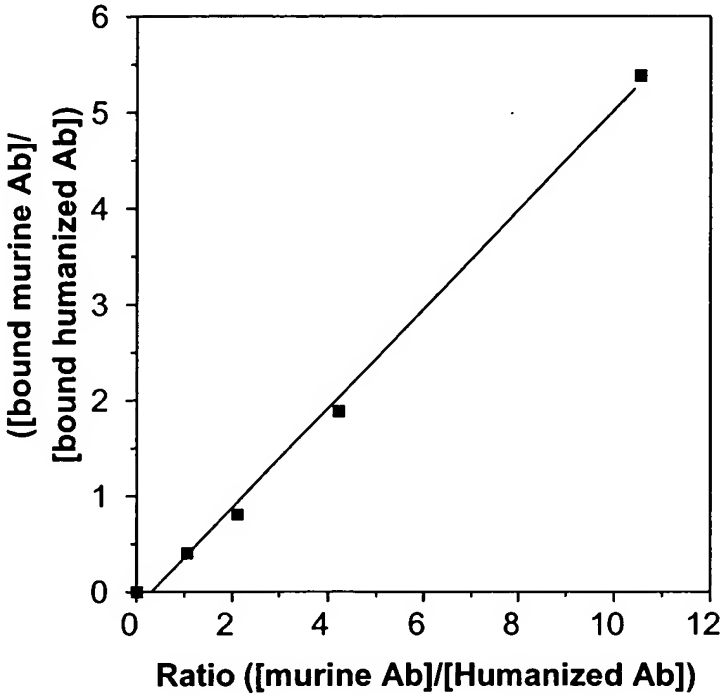


FIGURE 12

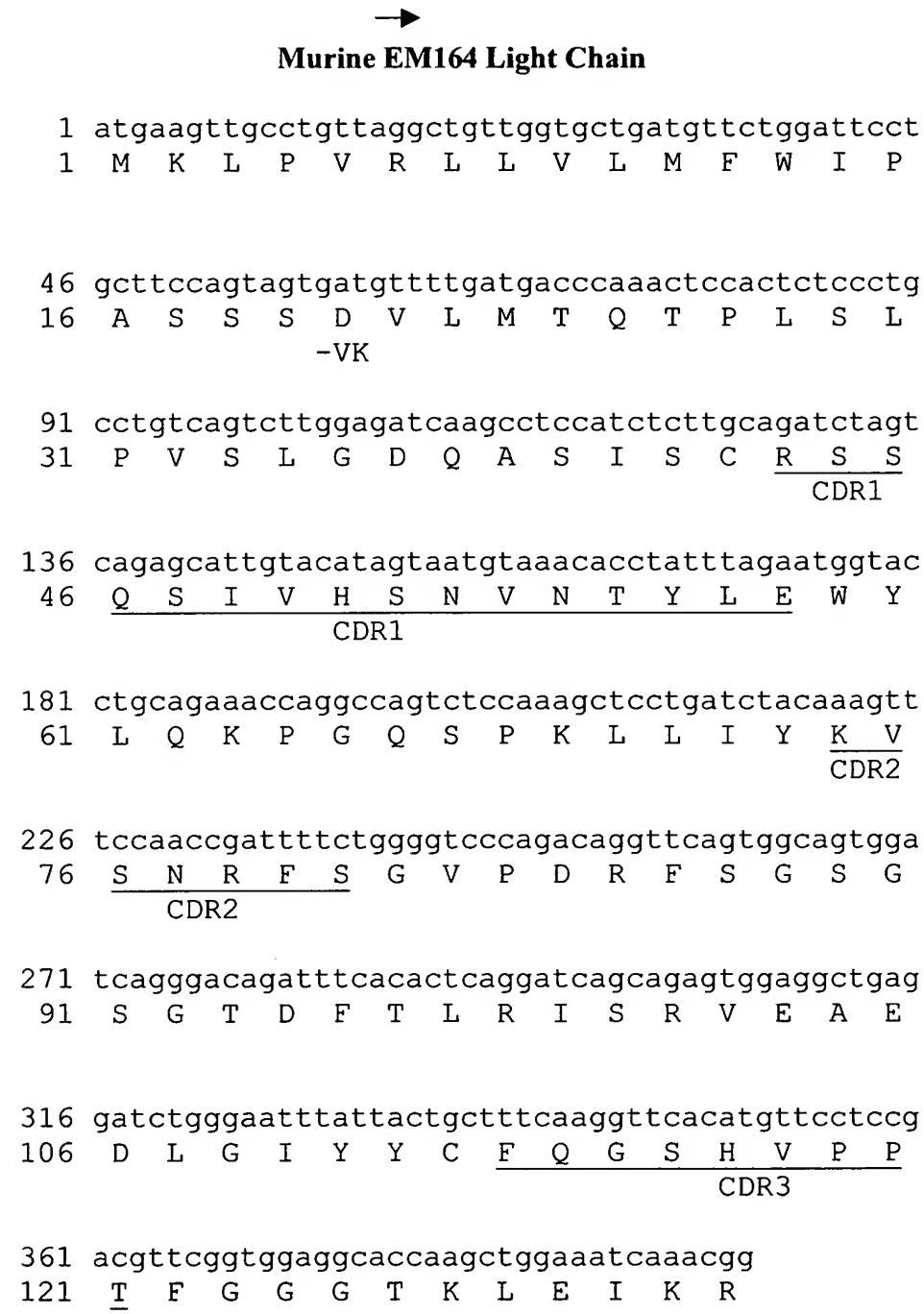

Murine EM164 Light Chain
1 atgaagttgcctgttaggctgttggtgctgatgttctggattcct
1 M K L P V R L L V L M F W I P
46 gcttccagtagtgatgttttgatgacccaaactccactctccctg
16 A S S S D V L M T Q T P L S L
-VK
91 cctgtcagtcttggagatcaagcctccatctcttgcagatctagt
31 P V S L G D Q A S I S C R S S
CDR1
136 cagagcattgtacatagtaatgtaaacacctatttagaatggtac
46 Q S I V H S N V N T Y L E W Y
CDR1
181 ctgcagaaaccaggccagtctccaaagctcctgatctacaaagtt
61 L Q K P G Q S P K L L I Y K V
CDR2
226 tccaaccgattttctggggtcccagacaggttcagtggcagtgga
76 S N R F S G V P D R F S G S G
CDR2
271 tcagggacagatttcacactcaggatcagcagagtggaggctgag
91 S G T D F T L R I S R V E A E
316 gatctgggaatttattactgctttcaaggttcacatgttcctccg
106 D L G I Y Y C F Q G S H V P P
CDR3
361 acgttcggtggaggcaccaagctggaaatcaaacgg
121 T F G G G T K L E I K R

FIGURE 13

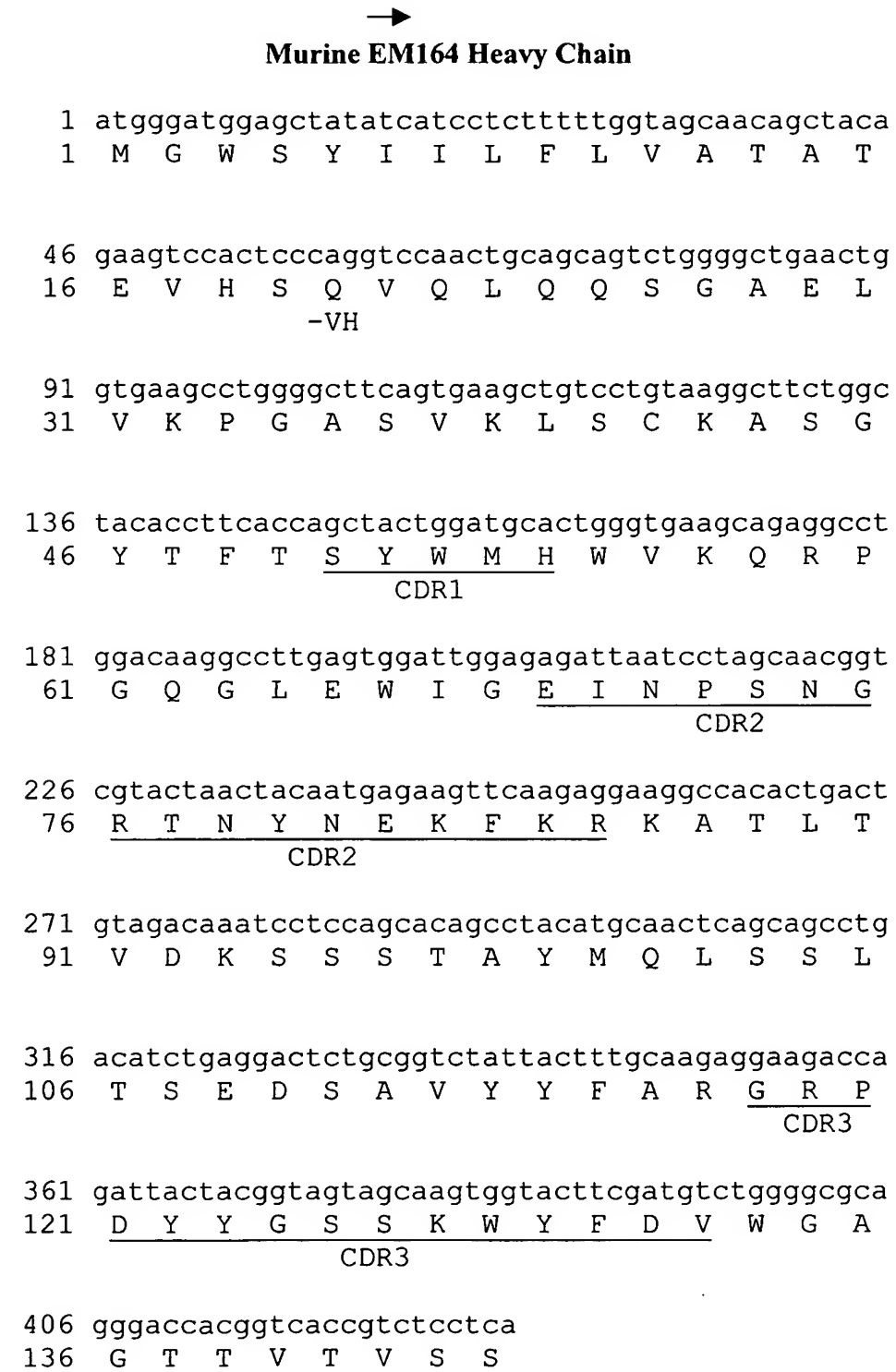

Murine EM164 Heavy Chain
1 atgggatggagctatatcatcctcttttggtagcaacagctaca
1 M G W S Y I I L F L V A T A T
46 gaagtccactcccaggtccaactgcagcagtctggggctgaactg
16 E V H S Q V Q L Q Q S G A E L
-VH
91 gtgaagcctggggcttcagtgaagctgtcctgtaaggcttctggc
31 V K P G A S V K L S C K A S G
136 tacaccttcaccagctactggatgcactgggtgaagcagaggcct
46 Y T F T S Y W M H W V K Q R P
CDR1
181 ggacaaggccttgagtggattggagagattaatcctagcaacggt
61 G Q G L E W I G E I N P S N G
CDR2
226 cgtactaactacaatgagaagttcaagaggaaggccacactgact
76 R T N Y N E K F K R K A T L T
CDR2
271 gtagacaaatcctccagcacagcctacatgcaactcagcagcctg
91 V D K S S S T A Y M Q L S S L
316 acatctgaggactctgcggtctattactttgcaagaggaagacca
106 T S E D S A V Y Y F A R G R P
CDR3
361 gattactacggtagtagcaagtggtacttcgatgtctggggcgca
121 D Y Y G S S K W Y F D V W G A
CDR3
406 gggaccacggtcaccgtctcctca
136 G T T V T V S S

FIGURE 14

Murine EM164 CDRs

Light Chain

CDR1: R S S Q S I V H S N V N T Y L E

CDR2: K V S N R F S

CDR3: F Q G S H V P P T

Heavy Chain

CDR1: S Y W M H

CDR2: E I N P S N G R T N Y N E K F K R

CDR3: G R P D Y Y G S S K W Y F D V

AbM Heavy Chain

CDR1: G Y T F T S Y W M H

CDR2: E I N P S N G R T N

CDR3: G R P D Y Y G S S K W Y F D V

FIGURE 15

Germline sequence comparisons

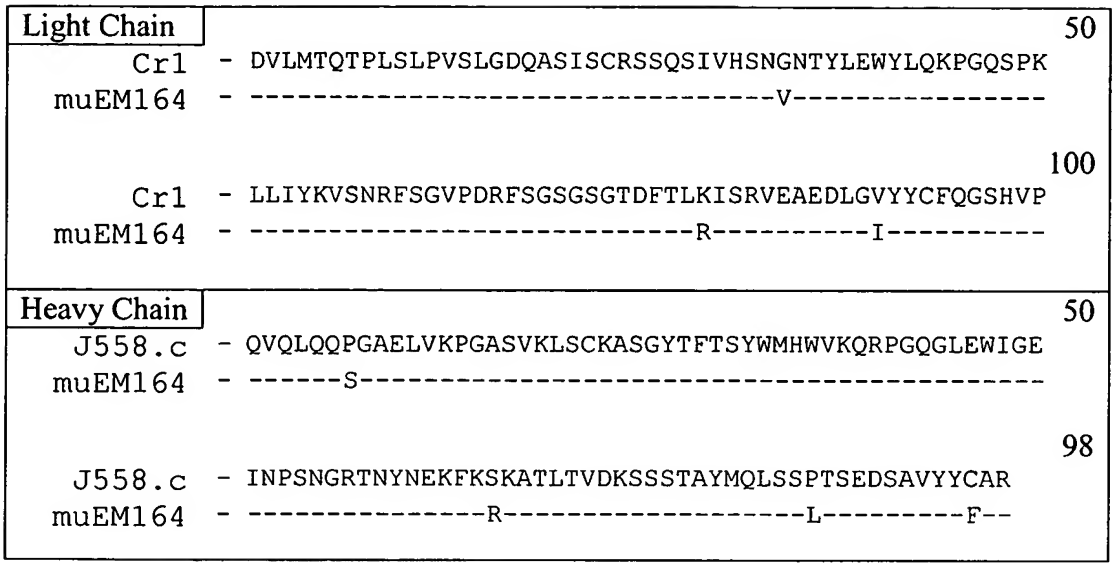


FIGURE 16

Cloning and Mammalian Expression Plasmid Maps

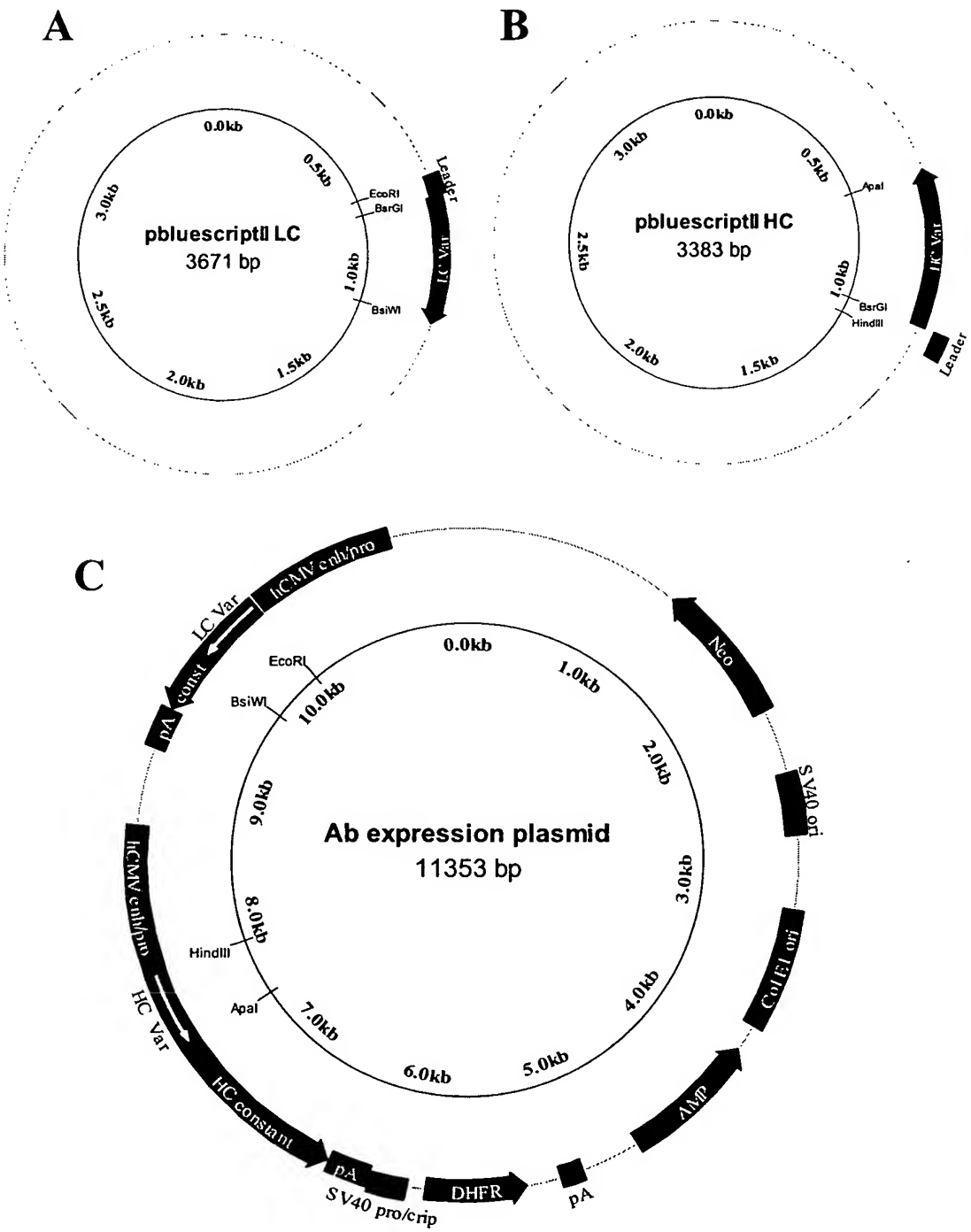


FIGURE 17

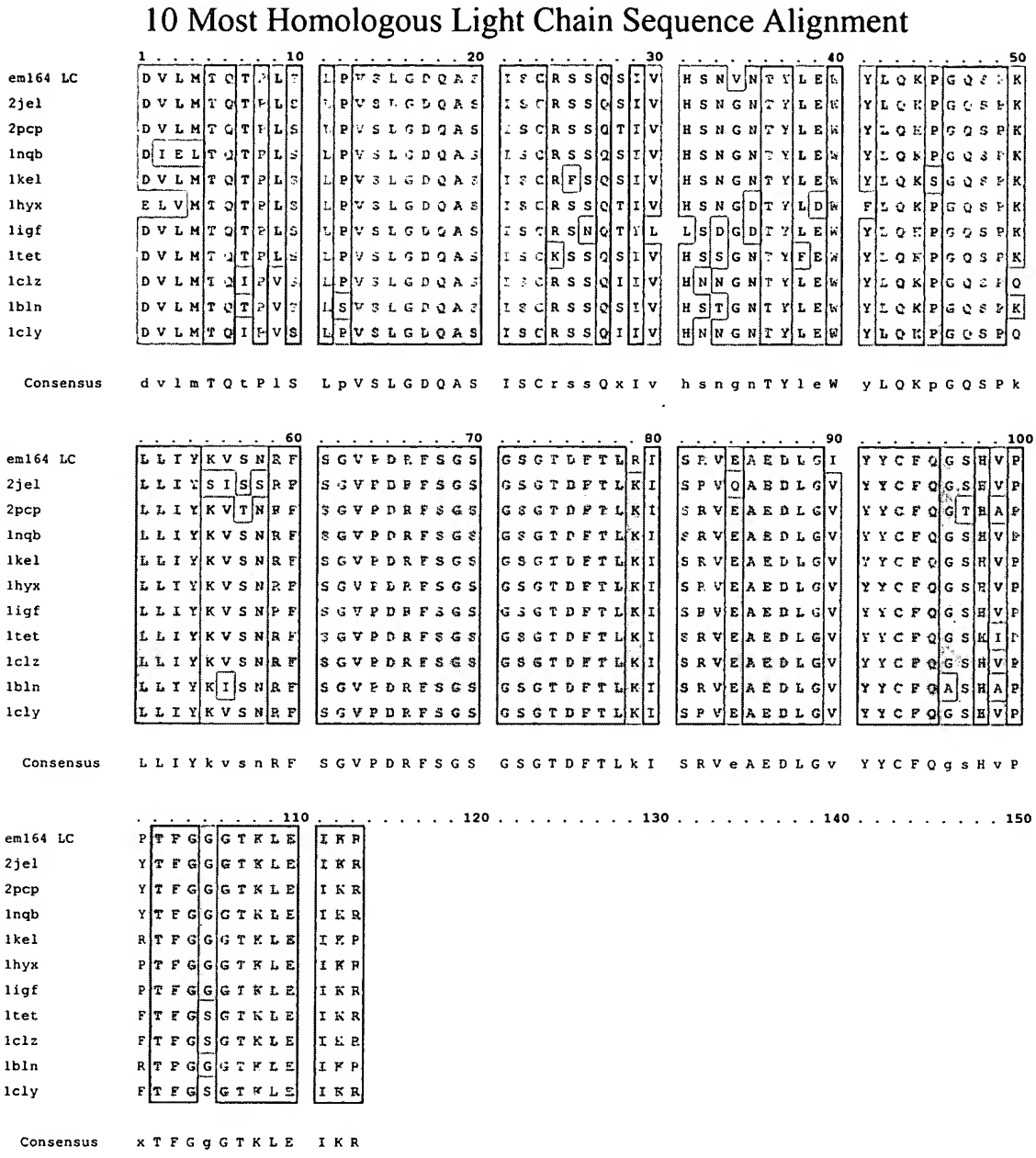

10 Most Homologous Light Chain Sequence Alignment
em164 LC
2jel
2pcp
1nqb
1kel
1hyx
1igf
1tet
1clz
1bln
1cly
Consensus
d v l m T Q t P l S L p V S L G D Q A S I S C r s s Q x I v h s n g n T Y l e W y L Q K p G Q S P k
Consensus
L L I Y k v s n R F S G V P D R F S G S G S G T D F T L k I S R V e A E D L G v Y Y C F Q g s H v P
Consensus
x T F G g G T K L E I K R

FIGURE 18

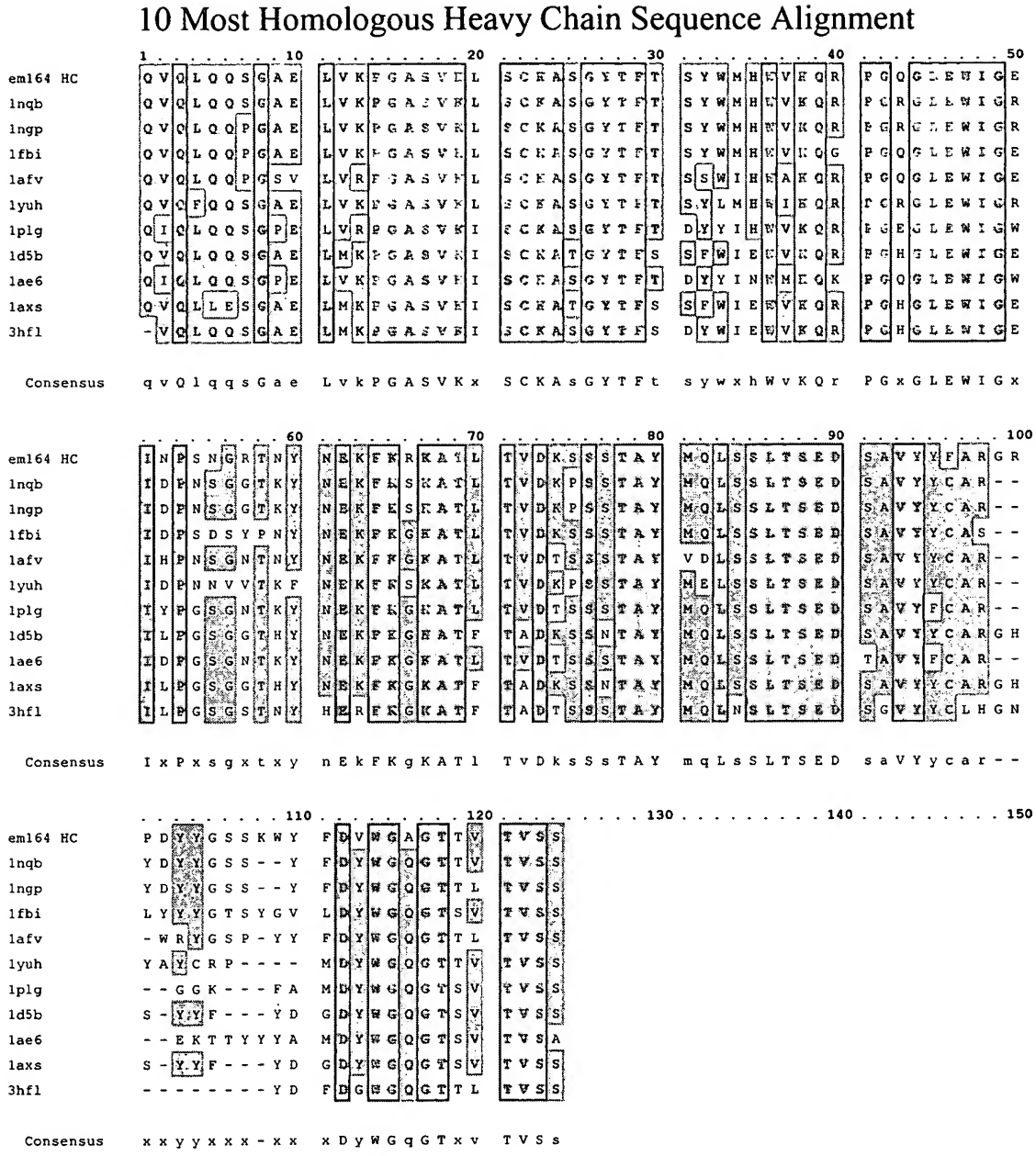
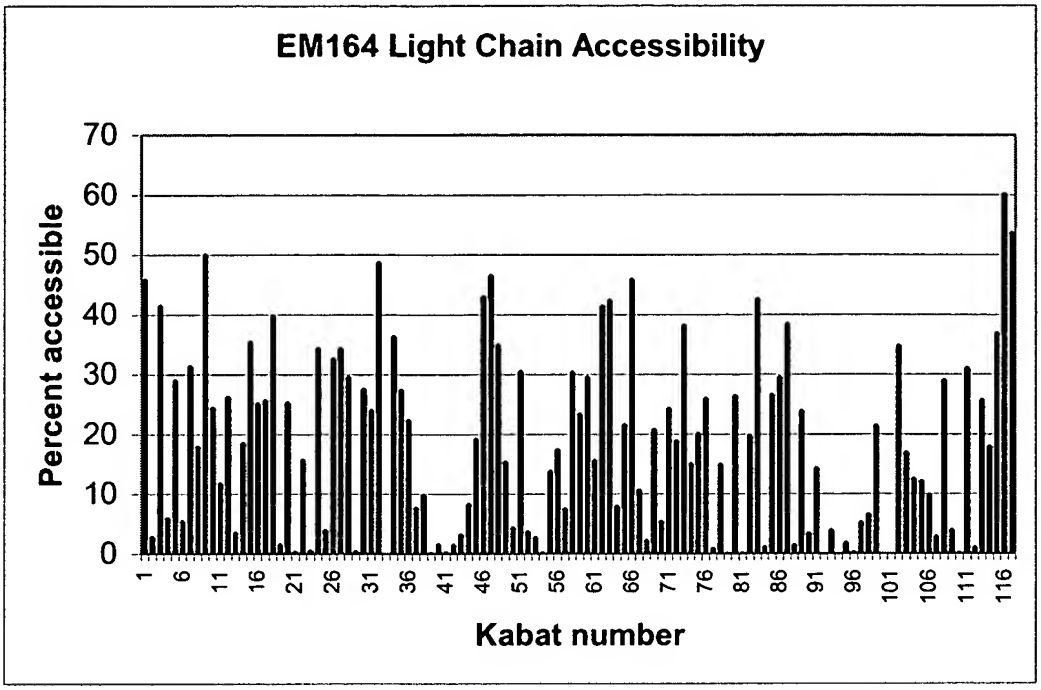

10 Most Homologous Heavy Chain Sequence Alignment
em164 HC
1nqb
1ngp
1fbi
1afv
1yuh
1plg
1d5b
1ae6
1axs
3hfl
Consensus

FIGURE 19

A.


EM164 Light Chain Accessibility
Percent accessible
0
10
20
30
40
50
60
70
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
76
81
86
91
96
101
106
111
116
Kabat number

B.

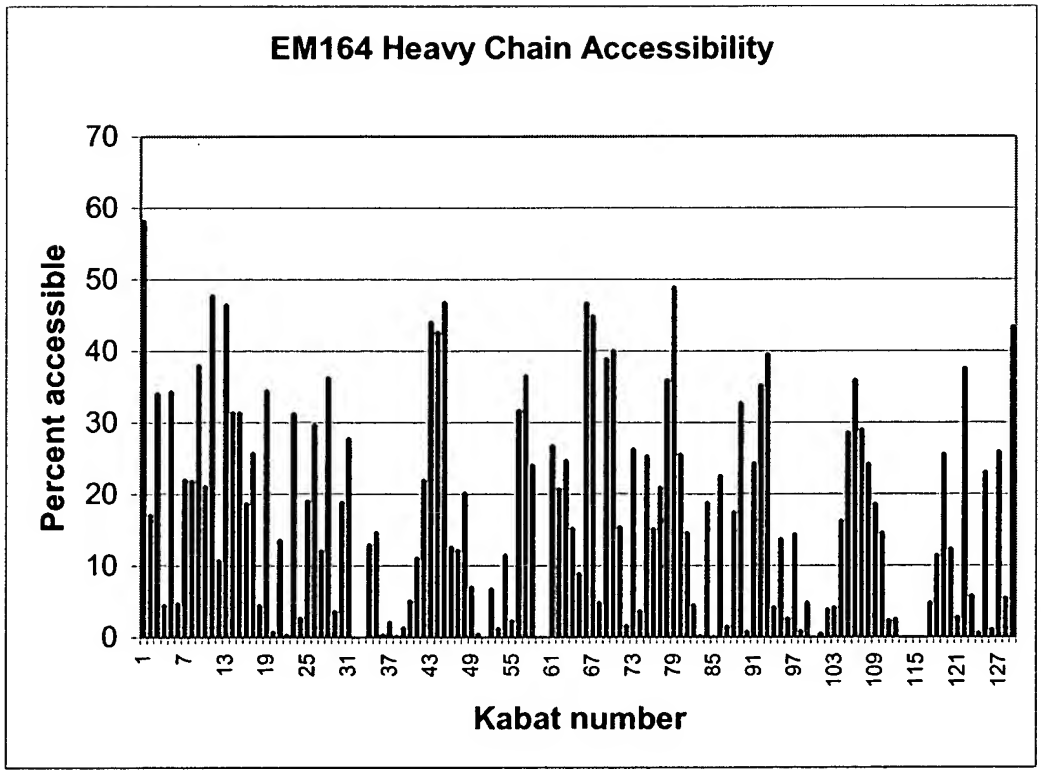

EM164 Heavy Chain Accessibility
Percent accessible
0
10
20
30
40
50
60
70
1
7
13
19
25
31
37
43
49
55
61
67
73
79
85
91
97
103
109
115
121
127
Kabat number

FIGURE 20

Light Chain Variable Region Amino Acid Sequences for Murine and Humanized EM164 Antibodies

```
Kabat #          1       10        20        27c       35        45
muEM164          DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV HSNVNTYLEW YLQKPGQSPK
huEM164 v1.0     DVVMTQTPLS LPVSLGDPAS ISCRSSQSIV HSNVNTYLEW YLQKPGQSPR
huEM164 v1.1     DVLMTQTPLS LPVSLGDPAS ISCRSSQSIV HSNVNTYLEW YLQKPGQSPK
huEM164 v1.2     DVLMTQTPLS LPVSLGDPAS ISCRSSQSIV HSNVNTYLEW YLQKPGQSPR
huEM164 v1.3     DVVMTQTPLS LPVSLGDPAS ISCRSSQSIV HSNVNTYLEW YLQKPGQSPK
changes            *               *                                  *

Kabat #          46      55        65        75        85        95f
muEM164          LLIYKVSNRF SGVPDRFSGS GSGTDFTLRI SRVEAEDLGI YYCFQGSHVP
huEM164 v1.0     LLIYKVSNRF SGVPDRFSGS GAGTDFTLRI SRVEAEDLGI YYCFQGSHVP
huEM164 v1.1     LLIYKVSNRF SGVPDRFSGS GAGTDFTLRI SRVEAEDLGI YYCFQGSHVP
huEM164 v1.2     LLIYKVSNRF SGVPDRFSGS GAGTDFTLRI SRVEAEDLGI YYCFQGSHVP
huEM164 v1.3     LLIYKVSNRF SGVPDRFSGS GAGTDFTLRI SRVEAEDLGI YYCFQGSHVP
changes                              *

Kabat #          96     105 108
muEM164          PTFGGGTKLE IKR
huEM164 v1.0     PTFGGGTKLE IKR
huEM164 v1.1     PTFGGGTKLE IKR
huEM164 v1.2     PTFGGGTKLE IKR
huEM164 v1.3     PTFGGGTKLE IKR
changes
```

FIGURE 21

Heavy Chain Variable Region Amino Acid Sequences for Murine and Humanized EM164 Antibodies

```
Kabat #   1       10        20        30        40        50
muEM164   QVQLQQSGAE LVKPGASVKL SCKASGYTFT SYWMHWVKQR PGQGLEWIGE
huEM164   QVQLVQSGAE VVKPGASVKL SCKASGYTFT SYWMHWVKQR PGQGLEWIGE
changes       *       *

Kabat #   51      59        69        79        86        96
muEM164   INPSNGRTNY NEKFKRKATL TVDKSSSTAY MQLSSLTSED SAVYYFARGR
huEM164   INPSNGRTNY NQKFQGKATL TVDKSSSTAY MQLSSLTSED SAVYYFARGR
changes              *   **

Kabat #   97     100f       109  113
muEM164   PDYYGSSKWY FDVWGAGTTV TVSS
huEM164   PDYYGSSKWY FDVWGQGTTV TVSS
changes                 *
```

FIGURE 22

huEM164 v1.0 Variable Region DNA and Amino Acid Sequences

Light Chain

```
  1 gatgttgtgatgacccaaactccactctccctgcctgtcagtctt
  1  D  V  V  M  T  Q  T  P  L  S  L  P  V  S  L

 46 ggagatccagcctccatctcttgcagatctagtcagagcatagta
 16  G  D  P  A  S  I  S  C  R  S  S  Q  S  I  V

 91 catagtaatgtaaacacctatttagaatggtacctgcagaaacca
 31  H  S  N  V  N  T  Y  L  E  W  Y  L  Q  K  P

136 ggccagtctccaaggctcctgatctacaaagtttccaaccgattt
 46  G  Q  S  P  R  L  L  I  Y  K  V  S  N  R  F

181 tctggggtcccagacaggttcagtggcagtggagcagggacagat
 61  S  G  V  P  D  R  F  S  G  S  G  A  G  T  D

226 ttcacactcaggatcagcagagtggaggctgaggatctgggaatt
 76  F  T  L  R  I  S  R  V  E  A  E  D  L  G  I

271 tattactgctttcaaggttcacatgttcctccgacgttcggtgga
 91  Y  Y  C  F  Q  G  S  H  V  P  P  T  F  G  G

316 ggcaccaaactggaaatcaaacgt
106  G  T  K  L  E  I  K  R
```

Heavy Chain

```
  1 caggtccaactggtgcagtctggggctgaagtggtgaagcctggg
  1  Q  V  Q  L  V  Q  S  G  A  E  V  V  K  P  G

 46 gcttcagtgaagctgtcctgtaaggcttctggctacaccttcacc
 16  A  S  V  K  L  S  C  K  A  S  G  Y  T  F  T

 91 agctactggatgcactgggtgaagcagaggcctggacaaggcctt
 31  S  Y  W  M  H  W  V  K  Q  R  P  G  Q  G  L

136 gagtggattggagagattaatcctagcaacggtcgtactaactac
 46  E  W  I  G  E  I  N  P  S  N  G  R  T  N  Y

181 aatcagaagttccaggggaaggccacactgactgtagacaaatcc
 61  N  Q  K  F  Q  G  K  A  T  L  T  V  D  K  S

226 tccagcacagcctacatgcaactcagcagcctgacatctgaggac
 76  S  S  T  A  Y  M  Q  L  S  S  L  T  S  E  D

271 tctgcggtctattactttgcaagaggaagaccagattactacggt
 91  S  A  V  Y  Y  F  A  R  G  R  P  D  Y  Y  G

316 agtagcaagtggtacttcgatgtctggggccaagggaccacggtc
106  S  S  K  W  Y  F  D  V  W  G  Q  G  T  T  V

361 accgtctcctca
121  T  V  S  S
```

FIGURE 23

huEM164 v1.1, 1.2, 1.3 Light Chain Variable Region DNA and Amino Acid Sequences

v1.1

```
  1 gatgttttgatgacccaaactccactctccctgcctgtcagtctt
  1  D  V  L  M  T  Q  T  P  L  S  L  P  V  S  L

 46 ggagatccagcctccatctcttgcagatctagtcagagcatagta
 16  G  D  P  A  S  I  S  C  R  S  S  Q  S  I  V

 91 catagtaatgtaaacacctatttagaatggtacctgcagaaacca
 31  H  S  N  V  N  T  Y  L  E  W  Y  L  Q  K  P

136 ggccagtctccaaagctcctgatctacaaagtttccaaccgattt
 46  G  Q  S  P  K  L  L  I  Y  K  V  S  N  R  F

181 tctggggtcccagacaggttcagtggcagtggagcagggacagat
 61  S  G  V  P  D  R  F  S  G  S  G  A  G  T  D

226 ttcacactcaggatcagcagagtggaggctgaggatctgggaatt
 76  F  T  L  R  I  S  R  V  E  A  E  D  L  G  I

271 tattactgctttcaaggttcacatgttcctccgacgttcggtgga
 91  Y  Y  C  F  Q  G  S  H  V  P  P  T  F  G  G

316 ggcaccaaactggaaatcaaacgt
106  G  T  K  L  E  I  K  R
```

v1.2

```
  1 gatgttttgatgacccaaactccactctccctgcctgtcagtctt
  1  D  V  L  M  T  Q  T  P  L  S  L  P  V  S  L

 46 ggagatccagcctccatctcttgcagatctagtcagagcatagta
 16  G  D  P  A  S  I  S  C  R  S  S  Q  S  I  V

 91 catagtaatgtaaacacctatttagaatggtacctgcagaaacca
 31  H  S  N  V  N  T  Y  L  E  W  Y  L  Q  K  P

136 ggccagtctccaaggctcctgatctacaaagtttccaaccgattt
 46  G  Q  S  P  R  L  L  I  Y  K  V  S  N  R  F

181 tctggggtcccagacaggttcagtggcagtggagcagggacagat
 61  S  G  V  P  D  R  F  S  G  S  G  A  G  T  D

226 ttcacactcaggatcagcagagtggaggctgaggatctgggaatt
 76  F  T  L  R  I  S  R  V  E  A  E  D  L  G  I

271 tattactgctttcaaggttcacatgttcctccgacgttcggtgga
 91  Y  Y  C  F  Q  G  S  H  V  P  P  T  F  G  G

316 ggcaccaaactggaaatcaaacgt
106  G  T  K  L  E  I  K  R
```

(FIGURE 23, CONT.)

v1.3

```
  1 gatgttgtgatgacccaaactccactctccctgcctgtcagtctt
  1  D  V  V  M  T  Q  T  P  L  S  L  P  V  S  L

 46 ggagatccagcctccatctcttgcagatctagtcagagcatagta
 16  G  D  P  A  S  I  S  C  R  S  S  Q  S  I  V

 91 catagtaatgtaaacacctatttagaatggtacctgcagaaacca
 31  H  S  N  V  N  T  Y  L  E  W  Y  L  Q  K  P

136 ggccagtctccaaagctcctgatctacaaagtttccaaccgattt
 46  G  Q  S  P  K  L  L  I  Y  K  V  S  N  R  F

181 tctggggtcccagacaggttcagtggcagtggagcagggacagat
 61  S  G  V  P  D  R  F  S  G  S  G  A  G  T  D

226 ttcacactcaggatcagcagagtggaggctgaggatctgggaatt
 76  F  T  L  R  I  S  R  V  E  A  E  D  L  G  I

271 tattactgctttcaaggttcacatgttcctccgacgttcggtgga
 91  Y  Y  C  F  Q  G  S  H  V  P  P  T  F  G  G

316 ggcaccaaactggaaatcaaacgt
106  G  T  K  L  E  I  K  R
```

FIGURE 24

Comparison of inhibition of IGF-I-stimulated growth and survival of MCF-7 cells by humanized EM164 v1.0 antibody (6-25 μg/mL) vs murine EM164 antibody (5-10 μg/mL)

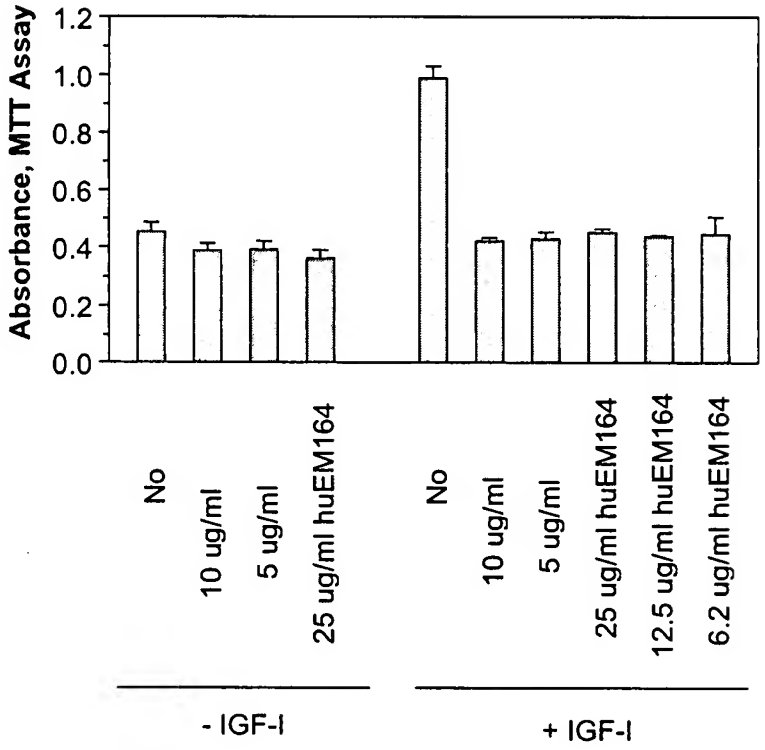


FIGURE 25

Cell cycle arrest of MCF-7 cells by EM164 antibody

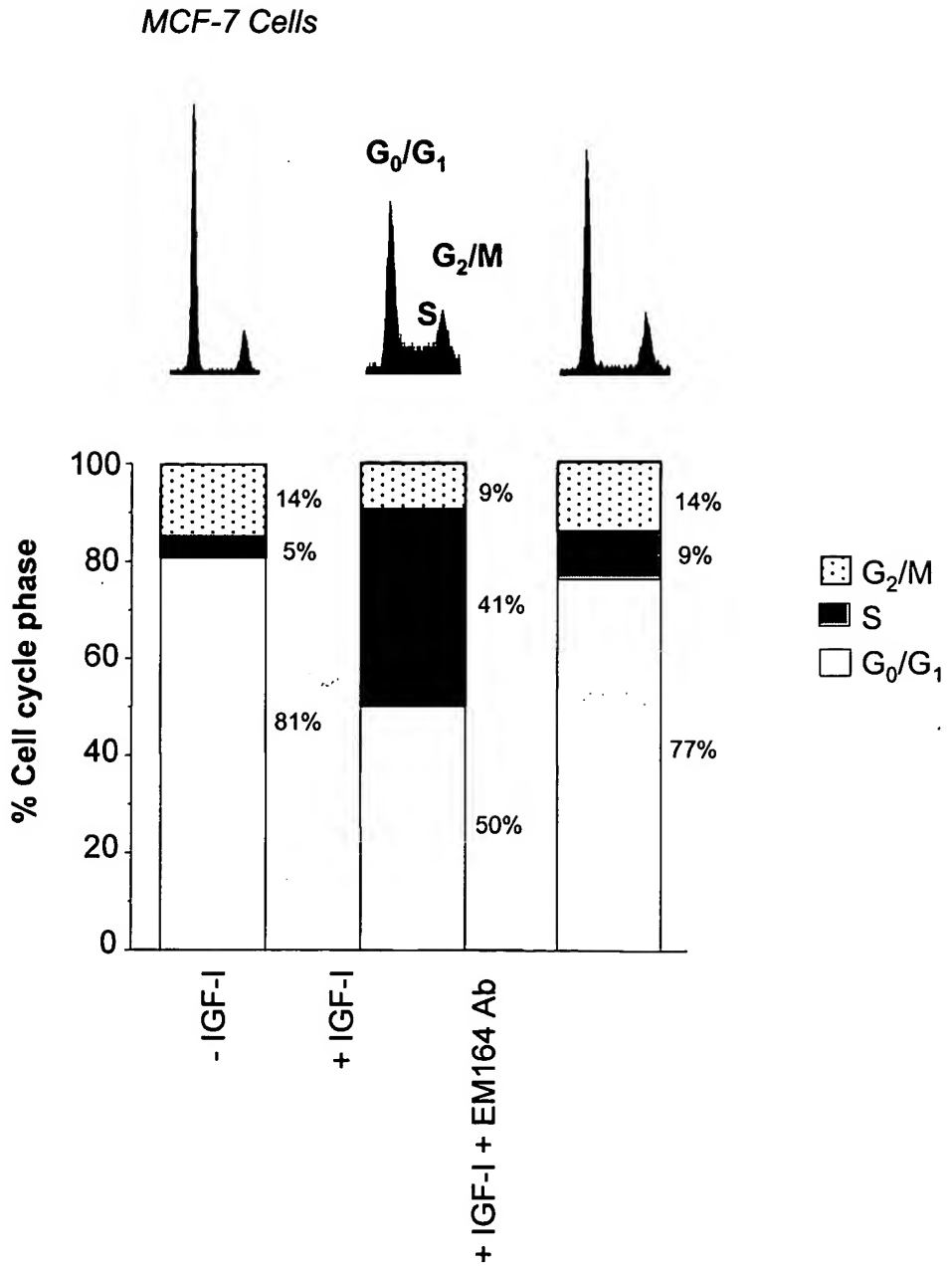


FIGURE 26

Induction of apoptosis in NCI-H838 lung cancer cells by EM164 antibody (measured from the cleavage of cytokeratin CK18 protein by caspase)

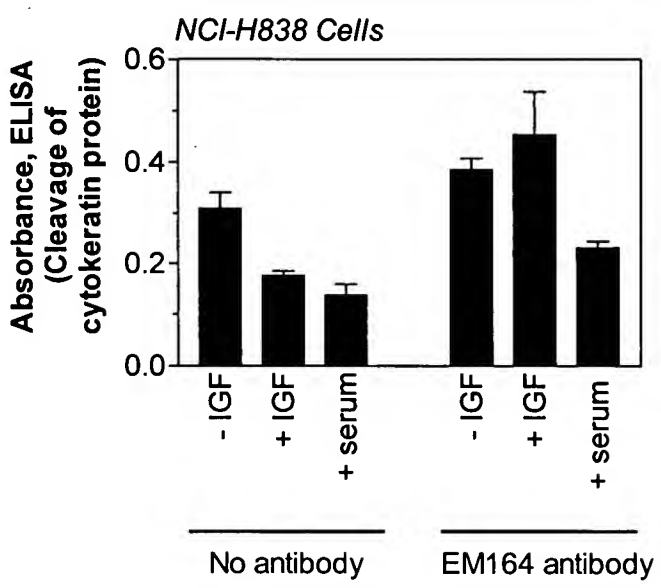


FIGURE 27

Suppression of growth of BxPC-3 human pancreatic cancer xenografts in mice by EM164 antibody treatment as a single agent or in combination with gemcitabine